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Elevation of cytokeratin 19 fragment (CYFRA 21-1) in serum of patients with radiation pneumonitis: possible marker of epithelial cell damage

J. Fujita[a,*], Y. Ohtsuki[b], S. Bandoh[a], H. Takashima[c], Y. Ueda[a], F. Wu[a], Y. Tojo[a], A. Kubo[a], T. Ishida[a]

[a]*First Department of Internal Medicine, Kagawa Medical University, Kagawa, 1750-1, Miki-cho, Kito-gun, Kawaga 761 0793, Japan*
[b]*Department of Pathology, Kochi Medical School, Kochi, Japan*
[c]*Department of Radiology, Kagawa Medical University, Japan*

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KEYWORDS
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Summary Cytokeratin 19 fragment (CYFRA 21-1) level in serum have already been documented as a useful tumor marker for lung cancer. In the present study, we hypothesized that CYFRA 21-1 increases in the sera of patients with radiation pneumonitis, resulting from epithelial cell damage. We measured CYFRA 21-1 in the sera of patients with radiation pneumonitis and evaluated the correlation between CYFRA 21-1 level and severity of radiation pneumonitis as well as clinical course. We studied 16 patients diagnosed with radiation pneumonitis associated with primary lung cancer. CYFRA 21-1 levels in the sera of patients with diffuse radiation pneumonitis ($n = 6$) significantly increased compared to normal smokers ($n = 10$) or patients with local radiation pneumonitis ($n = 10$). CYFRA 21-1 values in sera changed according to the progression or improvement of the diffuse radiation pneumonitis. An immunohistochemical study using pulmonary tissues obtained from autopsied patients with radiation pneumonitis demonstrated that the hyaline membrane and proliferating type II pneumocytes were strongly stained by the anti-human cytokeratin 19 antibody. Our data demonstrated that CYFRA 21-1 was increased in patients with diffuse radiation pneumonitis. Since CYFRA 21-1 is widely used as a tumor marker for lung cancer, this evidence should be noted especially in irradiated patients.

Introduction

There are approximately 40 different cell types in the lung.[1] Among them, the type II pneumocytes are the principal source of surfactant and have the fastest turnover rate. Since radiation injury is secondary to mitotic cell death, the type II pneumocyte is most closely linked to the mechanistic theories underlying radiation pneumonitis.[2] Pathophysiologically, it has been reported that radiation pneumonitis is an alveolitis resulting from damage to the alveolar type II pneumocytes that maintain alveolar patency.[2]

Cytokeratin is a cytoskeletal structure expressed only in epithelial cells.[3–6] More than 20 subtypes of

*Corresponding author. Fax: +81-87-891-2147.
E-mail address: jiro@kms.ac.jp (J. Fujita).

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cytokeratin are known. Of these cytokeratins, cytokeratin 19 is especially expressed in simple epithelia, including bronchial epithelial cells.[3–6] Although the precise mechanisms of their generation are unknown, cytokeratin 19 fragment(CYFRA 21-1) is soluble in serum and can be detected with the aid of monoclonal antibodies in patients with lung cancer.[7–10] However, there have been no studies evaluating the clinical significance of CYFRA 21-1 in sera of patients with radiation pneumonitis. Since it has been suggested that CYFRA 21-1 is released from injured bronchial epithelium,[11] we hypothesized that the levels of CYFRA 21-1 in sera also increase in patients with radiation pneumonitis, and CYFRA 21-1 values in sera correlate with the clinical course. Under this background, we measured CYFRA 21-1 levels in sera of patients with radiation pneumonitis.

Materials and methods

Subjects

In Kagawa Medical University Hospital (613 beds), we studied 16 patients with a diagnosis of radiation pneumonitis (1 female and 15 males; 2 ex-smokers and 14 current smokers). Their median age was 71 years with a range of 53–85 years.

The diagnoses of radiation pneumonitis were made by clinical, radiological, and physiological grounds. The criteria for diffuse radiation pneumonitis were defined as follows; (i) an interstitial shadow extending outside of irradiation field, (ii) a fall in arterial oxygen partial pressure of less than 60 mmHg in room air, (iii) absence of infectious pneumonia, and (iv) the need to administer steroid hormones to improve respiratory failure. Pneumonitis grading was defined using the National Cancer Institute Common Toxicity Criteria. NCI pneumonitis grading system defines Grade 1 as radiographic changes but asymptomatic or symptoms not requiring steroids. NCI Grade 2 pneumonitis is defined as radiographic changes and requiring steroids or diuretics. NCI Grade 3 pneumonitis is defined as radiographic changes and requiring oxygen. Grade 4 pneumonitis is for those with radiographic changes and requiring assisted ventilation. The localized radiation pneumonitis was defined as interstitial shadow within the irradiated field. In all patients, high-resolution computed radiographic scanning of the lungs (HRCT) was performed. Among 16 patients, 6 patients were classified to have diffuse radiation pneumonitis (grade 3 or 4) and 10 patients were classified to have focal radiation pneumonitis (all grade 1). Characteristics of 6 patients with diffuse radiation pneumonitis are listed in Table 1. CYFRA 21-1 were successively measured in 5 patients with diffuse radiation pneumonitis.

We also studied 10 normal smokers (smoking at least a pack/day, 9 men and 1 woman) who had an average age of 55 years. All subjects had no history of lung diseases or clinical findings suggesting lung disease, and all subjects had normal liver and renal function. They all had normal chest X-rays and their pulmonary function test results were within a normal range.

The ethics committees of the Kagawa Medical University approved the study, and all patients provided written informed consent before enrollment. The study was performed in accordance with the latest revisions to the Declaration of the Helsinki.

Blood samples

Peripheral venous blood samples with and without ethylenediaminetetraacetic acid (EDTA) were obtained before breakfast. After centrifugation at 1000*g* for 10 min at 4°C, the serum was frozen and stored at −70°C until used. Arterial blood samples were analysed for *P*aO2 and *P*aCO2 using a blood gas analyzer.

Measurement of CYFRA 21-1 levels in serum and correlation with clinical courses

CYFRA 21-1 in serum was measured with a two-step sandwich immunoassay using the streptavidin-biotin technique (Enzymun-Test CYFRA21-1; Boehringer Mannheim GmbH, Tutzing, Germany). The lower detection limit of this assay was 0.1 ng/ml. Data are expressed as mean values from duplicate determinations.

In patients with radiation pneumonitis, the correlation between CYFRA 21-1 and the clinical course was evaluated.

Pathological and immunohistochemical examination of the autopsied lung

To evaluate the expression of cytokeratin 19 in lung tissues obtained by autopsy. Paraffin embedded and dewaxed lung sections were immunohistochemically stained, employing the avidin-biotin peroxidase complex method (Dako LSAB kit-peroxidase, DAKO Corp., Kyoto, Japan) using mouse monoclonal antibody to cytokeratin 19; in 1:30 dilution, commercially purchased from ScyTek Laboratories (Logan, UT, USA). In order to enhance the immune

Table 1 Patient characteristics.

Case	Age and sex	Histology	Grade	Chemotherapy	Total dose (Gy)	Radition field (cm)	Treatment response
1	67 Male	Small cell lung cancer	4	CBDCA + ADM + VP-16	50.4 (1.2 Gy × 2/day)	10 × 8	PR
2	74 Male	Adenocarcinoma	4	None	60 (2 Gy × 1/day)	12 × 10	PR
3	71 Male	Small cell lung cancer	3	CBDCA + ADM + VP-16	55.2 (1.2 Gy × 2/day)	12 × 11	PR
4	53 Male	Adenocarcinoma	4	CBDCA + Taxotere	55.2 (1.2 Gy × 2/day)	10 × 10	PR
5	62 Male	Squamous cell carcinoma	4	CBDCA + Taxotere	50 (2 Gy × 1/day)	9 × 6	PR
6	85 Male	Squamous cell carcinoma	3	None	60 (2 Gy × 1/day)	7 × 5	PR

Interval between final radiotherapy and pneumonitis	Pattern	CYFRA (ng/ml)				Therapy	Prognosis
		Initial	Before	After	After therapy		
11	Diffuse	NE*	2.5	36.6	NE	Steroid	Dead†
2	Diffuse	NE	NE	45	NE	Steroid	Dead†
2	Diffuse	1	0.9	9.5	0.9	Steroid + Cyclosporin A	Improved
5	Diffuse	21.3	3.7	37.7	NE	Steroid	Dead
3	Diffuse	12.3	5	50	NE	Steroid + Cyclosporin A	Dead†
12	Diffuse	NE	2.9	6.7	NE	Steroid	Dead

*Not evaluable.
†Autopsy was performed.

reaction, dewaxed sections were pretreated with autoclave at 132°C for 12 min in 10 mM citrate buffer (pH 6.0). Staining procedures were according to the kit manual.

Histopathological findings of the lung of three autopsied cases were as follows. In case 1, each lobe of the lung was found to have organizing diffuse alveolar damage (DAD). The collapse of alveoli with fibrosis was more severe in the subpleural region, than in the deeper part, and irregular small multicystic changes associated with organized hyaline membranes were widely found. Proliferating type II alveolar cells with atypism were found in the intervening areas of small cystic change. In case 1, organizing DAD (acute interstitial pneumonia) was found in both lungs. A few remaining cancer cells were detected in the right lower lobe. The findings of case 2 and case 5, generally resembled case 1. Only a few remaining cancer cells were detected. All three autopsied cases were diagnosed to be bilateral radiation pneumonitis with a DAD pattern.

Statistical methods

Results are expressed as mean values ± standard error of the mean. Comparisons of values between the two groups were analysed with the Mann–Whitney *U*-test. A *P*-value less than 0.05 was considered to be statistically significant.

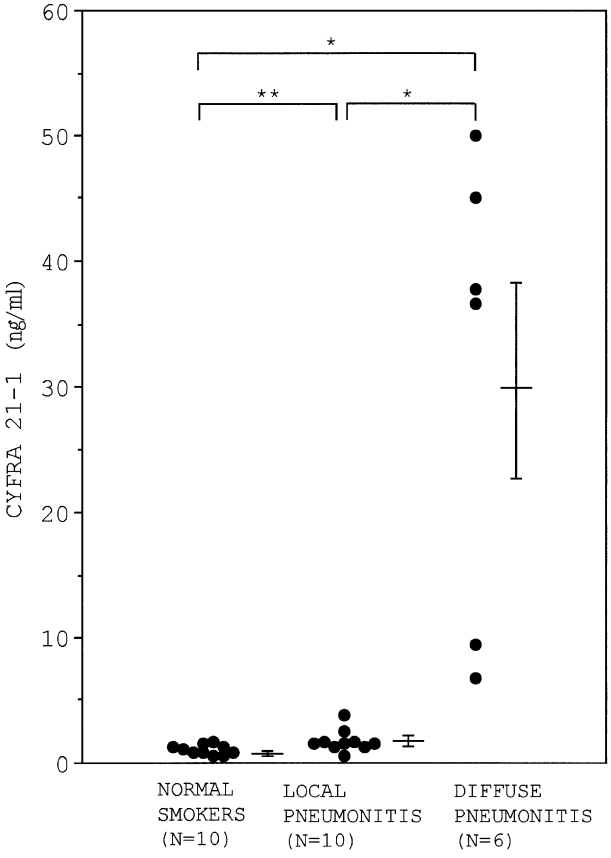

Figure 1 Serum cytokeratin 19 fragment levels (ng/ml) in normal smokers, patients with local radiation pneumonitis, and diffuse radiation pneumonitis. Bar represents mean + standard deviation. $^{*}P<0.002$, $^{**}P<0.02$.

Results

CYFRA 21-1 levels in sera of patients with focal radiation pneumonitis (1.74 ± 0.28 ng/ml, mean ± standard error) was significantly elevated as compared with normal smokers (1.0 ± 0.13 ng/ml) ($P<0.02$) as shown in Fig. 1. In addition, CYFRA 21-1 levels in sera of patients with diffuse radiation pneumonitis (30.9 ± 7.5 ng/ml, mean ± standard error) were significantly elevated as compared with local radiation pneumonitis (1.74 ± 0.28) and normal smokers (1.0 ± 0.13 ng/ml) ($P<0.002$ in both comparisons). Serum CYFRA 21-1 levels in patients with radiation pneumonitis were changed according to the treatment as shown in Fig. 2A and B. Patients were treated with the pulse therapy of intravenous methylprednisolone (1 g/day for 3 days) and followed by oral prednisolone (1 mg/kg). In patients with deteriorated radiation pneumonitis (case 1, 4 and 5), CYFRA 21-1 levels increased according to the clinical course (Fig. 2A. In addition, in a case (case 3) in whom radiation pneumonitis was improved by treatment, CYFRA 21-1 values decreased according to the clinical course (Fig. 2B). Interestingly, CYFRA 21-1 levels in serum did not correlate with CRP values in sera (Fig. 3).

Immunostaining of the hyaline membrane with anti-human cytokeratin 19 monoclonal antibody was positive in an autopsy case of radiation pneumonitis as shown in Fig. 4A, featuring somewhat lamellar structure. Detached epithelial cells were also stained (Fig. 4A). Proliferating type II pneumocytes lining irregular alveolar spaces were stained much more densely than the hyaline membrane, as revealed in Fig. 4B. Cytoplasm of CD68 positive intraalveolar macrophages was also weakly stained (Fig. 4C).

Discussion

In the present study, it was demonstrate that the CYFRA 21-1 levels in sera of patients with diffuse radiation pneumonitis were significantly higher as compared with local radiation pneumonitis or

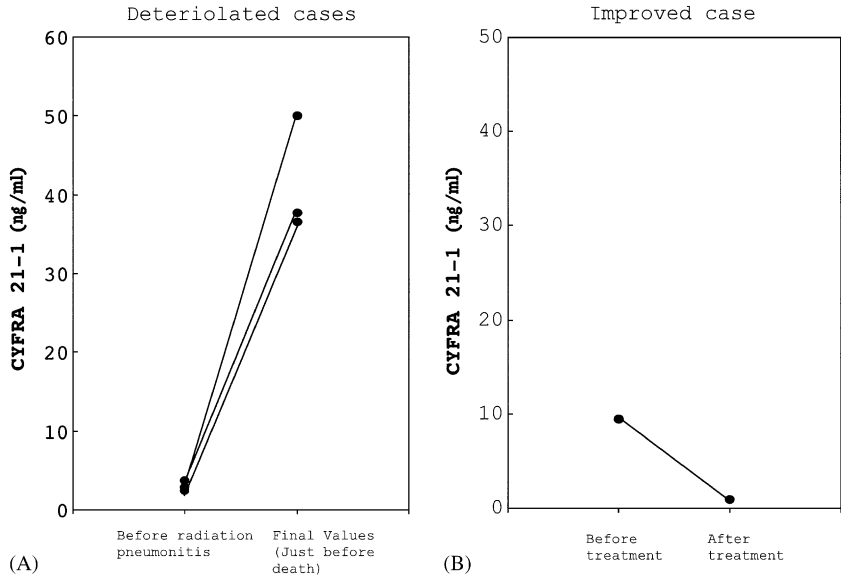

Figure 2 Changes of serum cytokeratin 19 fragment levels (ng/ml) according to clinical courses in 5 patients with diffuse radiation pneumonitis. (A) Deteriorated cases; (B) an improved case. In patients with radiation pneumonitis, CYFRA 21-1 levels increased according to the progressing clinical course (A). In contrast, a case in which radiation pneumonitis had improved by treatment, CYFRA 21-1 levels decreased according to the improving clinical course (B).

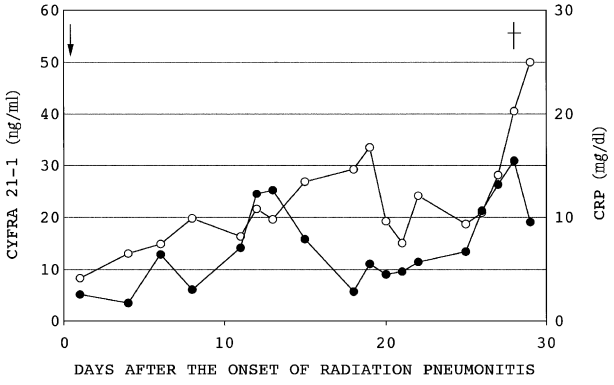

Figure 3 Changes of serum CYFRA 21-1 levels (ng/ml, ○) and CRP values (mg/dl, ●) along with the clinical course of a patient with diffuse radiation pneumonitis. The arrow represents the onset of radiation pneumonitis.

normal smokers, and CYFRA 21-1 levels in sera were significantly correlated with the clinical course in patients with radiation pneumonitis. In addition, CYFRA 21-1 values in sera of patients with deteriorating radiation pneumonitis were extraordinarily high. Furthermore, immunohistochemical staining demonstrated that cytokeratin 19 was stained in moderate density at the site of the hyaline membrane. This evidence suggests that CYFRA 21-1 levels in serum reflected the disease activity in patients with radiation pneumonitis, and CYFRA 21-1 was a useful marker to prove the existence of epithelial cell damage.

The accepted explanation for development of radiation pneumonitis has largely centered around the process of cellular damage, particularly the type II pneumocytes, and the resultant alteration in production and release of pulmonary surfactant that is needed to prevent atelectasis of the lung.[12] In radiation induced lung injury, it has been suggested that apoptosis of type II pneumocytes plays an important role.[13] Recently, a review article about lung toxicity following chest irradiation in patients with lung cancer[14] and several original articles concerning the pathogenesis of radiation-induced lung injury have been published.[15–18]

It has been suggested that CYFRA 21-1 is a marker of apoptosis in several human cancers.[19–21] Therefore, following cell injury caused by radiation, fragments of cytokeratin 19 might be released from type II pneumocytes during the process of radiation-induced epithelial cell damage. In addition, a part of cytokeratin 19 derived from damaged or apoptotic type II pneumocytes might be a component of hyaline membrane.

As demonstrated in the present study, successive measurement of serum CYFRA 21-1 and CRP resulted in the increased value of serum CYFRA 21-1, but not CRP. CRP is produced from hepatocyte by the stimulation of interleukin-1, interleukin-6, and TNF-α. These cytokines were suppressed by corticosteroids, and then CRP decreased immediately after the steroid treatment. In contrast, epithelial cell damage as well as successive repair by alveolar type II cells may have reflected the

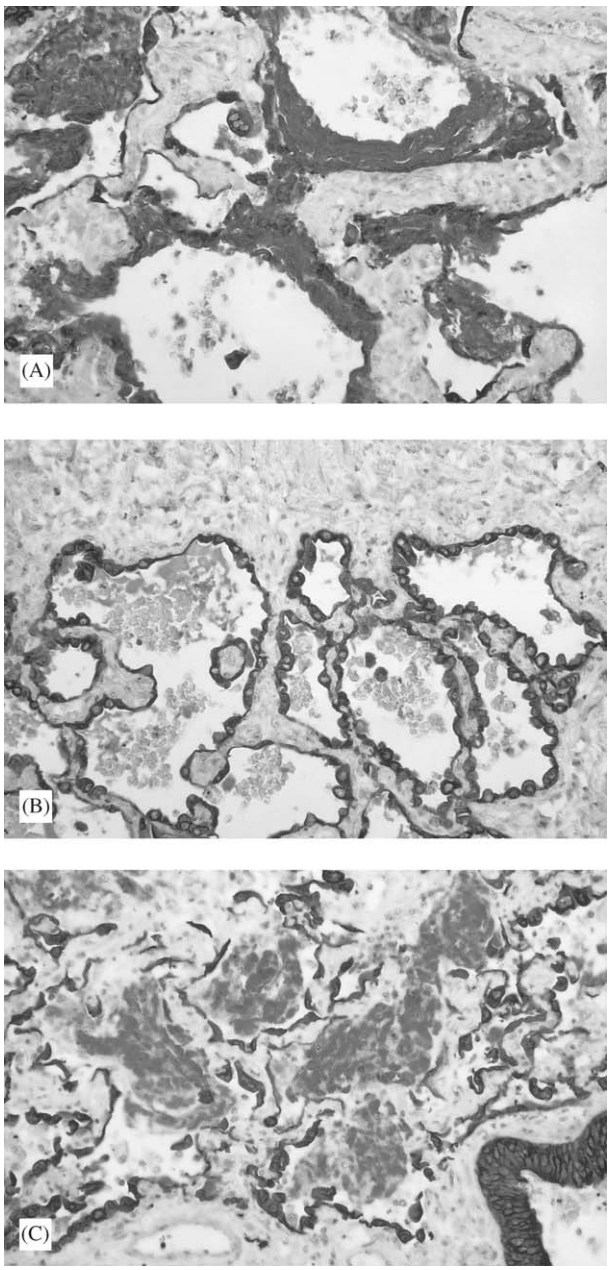

Figure 4 Immunohistochemical staining with anti-human cytokeratin 19 monoclonal antibody in a patient with diffuse radiation pneumonitis. (A) The hyaline membrane is strongly positive with this antibody. In addition, detached epithelial cells are also positive. Avidin-biotin-peroxidase complex method, $\times 220$. (B) Cytoplasm of proliferating type II pneumocytes are strongly positive. Avidin-biotin-peroxidase complex method, $\times 220$. (C) Alveolar macrophages, positive for CD68 are also weakly stained. Note densely stained bronchiolar epithelial layer. Avidin-biotin-peroxidase complex method, $\times 220$.

increase of CYFRA 21-1. Therefore, there may have been a discrepancy between the CRP values and CYFRA 21-1 values.

Since CYFRA 21-1 levels in sera frequently increase in patients with lung cancer, elevation of CYFRA 21-1 might be caused by the progression of lung cancer itself (for example, lymphangitis carcinomatosa). However, in the present study, lung cancer was well controlled in most patients clinically, the possibility of the progression of lung cancer was very low. In addition, in 3 autopsied cases, lung cancer was well controlled and the mass of remnant tumor was too small to explain values of serum CYFRA 21-1. Therefore, the possibility of lymphangitis carcinomatosa was not considered by either clinical findings or autopsy findings.

Although there are some limitations for measuring CYFRA 21-1 in lung cancer patients, it should be stressed that levels of CYFRA 21-1 in sera could increase in patients with radiation pneumonitis. In addition, since CYFRA 21-1 was only released from injured epithelial cells, levels of CYFRA 21-1 directly reflected the degree of epithelial cell damage as demonstrated in immunohistochemistry.

Since the present study is retrospective, a prospective study evaluating the usefulness of CYFRA 21-1 measurement in patients who receive radiation therapy should be performed.

In conclusion, our data demonstrate (i) CYFRA 21-1 in serum increased in patients with diffuse radiation pneumonitis, and (ii) CYFRA 21-1 in serum correlated with the clinical course in radiation pneumonitis. These results suggest that the elevation of CYFRA 21-1 seemed to be a marker of lung epithelial cell damage, possibly caused by apoptosis of epithelial cells from radiation pneumonitis. Physicians should also consider that the elevation of CYFRA 21-1 was also caused by radiation pneumonitis instead of recurrence of primary lung cancer.

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